means for detecting any carbohydrate-free transferrin.

REMARKS

Applicants have carefully reviewed and considered the Advisory Action mailed on September 4, 2002, and the reference cited therewith. Reconsideration and withdrawal of the rejections of the claims, in view of the amendments and remarks presented herein, is respectfully requested.

Claims 1, 3-12 have been amended; new claims 13-17 have been added; as a result, claims 1-17 are now pending in this application.

Support for new claims 13-17 are as follows:

independent claim 13, is found, for example, on page 23, lines 1-31, and Example 8;

dependent claim 14, is found on page 21, lines 1-31;

dependent claim 15, is found on page 23, lines 1-31;

dependent claim 16, is found on page 23, lines 1-31; and

dependent claim 17, is found on page 21-23, and Examples 7 and 8.

The following headers and numbered paragraphs correspond to those in the official action.

Claim Objections

3. Claims 3-12 under 37 CFR 1.75(c) were objected to as being in improper dependent form. Applicant previously amended the improper dependencies by a preliminary amendment. A copy of the preliminary amendment and the date stamped postcard is enclosed herewith. Accordingly, the objection under 37 CFR 1.75(c) is believed to be overcome.

Claim Rejections –35 USC §112

4. Claims 3 and 5 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. The rejection is respectfully traversed.

In particular, the Examiner asserts that claims 3 and 5 are vague and indefinite for the phrases "fragments thereof" and "mixtures thereof".

It is well-settled that claim language is sufficiently definite if one of ordinary skill in the art would understand the scope of the claim when read in light of the specification. In re Marosi, 710 F.2d 799, 218 U.S.P.Q. 289 (Fed. Cir. 1983); Morton Inst. Inc. v. Cardinal Chemical Co., 28 U.S.P.Q.2d 1190 (Fed. Cir. 1993); Miles Laboratories v. Shandon Inc., 27 U.S.P.Q.2d 1123 (Fed. Cir. 1993), *cert. denied*, 510 U.S. 1100 (1994).

Applicants have amended claim 3 to remove "thereof" and to leave "fragments" which is supported in the specification as filed, for example, on page 9, lines 28-31, and where examples of "antibody fragment" are provided. Antibody "fragments" are well known in the art, see for example, Sundrehagen's (WO 91/19983) working Examples 4-6, pages 19-22, which describe the preparation, purification, and use of labeled antibody fragments (Fab) derived from IgG, such as by digestion of IgG antibodies.

Claims 3 and 5 were both amended to include "selected from the group consisting of" and "and mixtures thereof" which is believed to be a proper claim format permitted under the rules, see M.P.E.P. § 2173.05(h). The application is directed to one of ordinary skill in the art and as such the claims are concise and definite to one skilled in the art given the examples of "combination", for example, on page 9 (lines 9-14), such as "any combination" and "one or more" carbohydrate-binding ligands; and page 10 (lines 7-9) and in view of the ordinary meaning of "mixture" and "thereof" (e.g. Merriam-Webster Collegiate

Dictionary). In the present context "mixtures thereof" is readily understood by one skilled in the art to mean combinations of the preceding enumerated group members, i.e. one or more carbohydrate-binding ligands. The common characteristics of the members of the recited groups of claims 3 and 5 is that the members are "carbohydrate-binding ligands," that is, ligands which are capable of binding carbohydrate molecules when used individually, or in combinations. The recited members are readily recognized by those of ordinary skill in the art as suitable carbohydrate-binding ligands and as described in the present invention. Applicant discloses that one or more carbohydrate-binding ligands, i.e., generally a protein capable of binding to any carbohydrate, oligosaccharide or sugar structures, may be employed to separate carbohydrate-free transferrin (CFT) from other transferrin variants (page 9, lines 19-28). For example, the carbohydrate-binding protein can be an antibody, e.g., a monoclonal antibody, a polyclonal antibody, an antibody fragment, single chain antibody, or a lectin "used singularly or in combination with other types of carbohydrate-binding proteins" (emphasis added, page 10, lines 7-10 and page 9, lines 28-36). Combinations of different carbohydrate-binding ligands are disclosed to have increased binding capacity and hence provide better separation of transferrin isoforms (page 11, lines 3-11). Examples of such combinations are disclosed at page 12, lines 13-22.

Thus, it is clear from the claims and the specification that "mixture thereof" refers to a combination of one or more carbohydrate-binding ligands. Hence, one of ordinary skill in the art, in possession of the specification, would be readily apprised of the metes and bounds of "mixtures thereof" in context of the present claims.

Accordingly, withdrawal of the rejection of the claims 3 and 5 under §112, second paragraph, is respectfully requested.

5. Claims 1-12 were rejected under 35 U.S.C. § 112, second paragraph, as being incomplete for allegedly omitting essential steps. The rejection is respectfully traversed.

The Examiner maintained his previous rejection of claims 1-12 under 35 U.S.C. § 112, second paragraph, as being "incomplete for allegedly omitting essential steps . . . amounting to a gap between the steps" which conclusion Applicant disagrees. In particular, the Examiner asserts that all method steps must be positively recited in the claims (citing Ex Parte Erlich 3 USPQ 101 [sic, 3 USPQ2nd 1011]). The Examiner's reliance on Erlich is misguided since claims 6 and 7 at issue in Erlich were not in an active 'gerund' form contrary to the claims of the present invention. Erlich in relevant part (§ 112, 2nd paragraph rejections) stands for the proposition that process steps need not recite all of the operating details but should recite a positive, active step(s) so that the claim will 'set out and circumscribe a particular area with a reasonable degree of precision and particularity'(citing In re Moore), making it clear what subject matter the claims encompass (citing In re Hammack), as well as making it clear the subject matter from which others would be precluded (citing In re Hammack). Ex Parte Erlich at 1017. It is also well settled that in a multi-step process which is an improvement of a conventional process, it is not necessary to define in detail the conventional steps of the claimed process which are not the improved steps. Ex parte Davidson et al., 116 USPQ 529 (POBA 1957). A conventional process is, for example, a prior art process for determining carbohydrate deficient transferrin (CDT), such as, Applicant's WO 96/26444 application cited by the Examiner. In the present process the novelty and unobviousness reside in the combination of specific sample manipulation steps, and their sequence or ordering, affording a simple and efficient determination which is highly specific for the carbohydrate free transferrin (CFT) content in body fluids, see for example claim 1. Additionally, the specification is clear that determining the presence of any CFT with the present method can be indicative of alcohol abuse, see for example claims 13 and 14. The less specific prior processes, such as WO 96/26444, which measured carbohydrate deficient transferrin CDT, provided at best, a correlation or approximate ranges (with overlap) among alcohol abuser, non-abuser, and abstainer groups (see for example, WO 96/26444, Example 13, page 29). The

active, positive steps recited in Applicant's method claim 1, include a) contacting; b) separating and contacting; and c) determining, and are defined in detail in the application as filed. The recited steps are readily apparent to one of ordinary skill in the art. Additionally, the individual steps of Applicant's recited methods are versatile in that they can be accomplished with considerable flexibility so that the methods are amenable to differences in the availability of materials and detection means, such as sample matrix, ligands, spectrophotometers, chromatographic supports, instrumentation, direct versus indirect determination of the analyte, and the like.

The Examiner suggested that the Applicant's "determining step" of claim 1 (c) must include "contacting" and "detecting" steps which Applicant believes to be unnecessary for the reasons of record and as discussed above and below. Applicant reiterates that it is not necessary to contact the CFT with anything in order to quantify CFT. Although Applicant respectfully points out that claim 1, step (a) recites "contacting". As disclosed at page 19, lines 8 to 22, of the specification, the content of transferrin in the non-binding or "CFT containing" fraction may be determined either by directly assessing the transferrin content of the separated fraction, or alternatively, an indirect determination may be carried out in which the transferrin content of the bound fraction is determined and then subtracted from the total transferrin content of the initial sample in order to find the CFT content. Many different standard procedures are known in the art for carrying out such determination, e.g., ELISA or radio immunoassay techniques, and it is therefore inappropriate to limit claim 1 to a particular CFT determination. Applicant's specification discloses that CFT, that is the transferrin which is completely devoid of carbohydrate side chains and is substantially free of any residual N-linked oligo-saccharides, is an indicator or marker for alcoholism (see page 6, lines 28-33). Any assay known for detecting and/or quantifying transferrin can be used to determine the CFT content of a sample, for example by difference, which Applicant discloses is sufficient for a clinically valuable assessment of alcoholism (see page 6, lines 34-38). For example, Applicant

discloses that assays such as an ELISA, radioimmunoassay, radioimmodiffusion, rocket immunoelectrophoresis, or particle-based immunoassay may be employed, or other assays known to the art such as those disclosed in U.S. Patent No. 4,626,355 (see page 19, lines 16-35). Applicant discloses in the working examples the detection of CFT in physiological samples using anti-transferrin antibodies (see Examples 1-5 and 7-8). To satisfy the requirements of 35 U.S.C. §112, second paragraph, it is well-settled that Applicant need not recite every assay which can be used to detect CFT by Applicant's method. In re Angstadt, 190 U.S.P.Q. 214, 218 (C.C.P.A. 1976); Ex parte Dubbs and Stevens, 119 U.S.P.Q. 440 (Bd. App. 1958). The Examiner's attention is respectfully directed to page 21, lines 24-26, of the specification. "Correlation" of the amount of CFT is a diagnostic step, not a step of the assay method as claimed. The method of claim 1 of the present invention is an assay which allows a determination of CFT content to be made. As disclosed on page 21 of the specification, "it may be assumed that the presence of any CFT whatsoever is indicative of alcohol abuse," but this is a matter for the medical practitioner to assess in the context of each individual patient.

Based on the above remarks, it is respectfully submitted that the pending claims comply with 35 U.S.C. § 112, second paragraph. Thus, withdrawal of the rejection of the is respectfully requested.

—> Claim Rejections - 35 U.S.C. § 102(b)

6. The Examiner maintained the rejection claims 1-3, 6-8 and 10-12 under 35 U.S.C. § 102(b) as being anticipated by Sundrehagen WO 91/19983 ("Sundrehagen"). As this rejection may be maintained with respect to the pending claims, it is respectfully traversed.

The standard for anticipation is one of strict identity, and to anticipate a claim for a patent a single prior art source must contain all its elements. Hybritech Inc. v. Monoclonal Antibodies, Inc., 231 U.S.P.Q.2d 90 (Fed. Cir. 1986); In re Dillon, 16 U.S.P.Q.2d 1987 (Fed. Cir. 1990). Furthermore, there

must be no difference between the claimed invention and the disclosure, as viewed by a person of ordinary skill in the art. Scripps Clinic & Res. Found. v. Genentech, Inc., 18 U.S.P.Q.2d 1001 (Fed. Cir. 1991).

Sundrehagen discusses a method of separating different transferrin variants, particularly CDT (carbohydrate deficient transferrin) from "normal" carbohydrate-containing variants. In that method, fractionation techniques (such as using the different isoelectric points of the different transferrin variants) are used to separate these transferrin variants and a binding partner is then used to label the separated variants. However, Sundrehagen does not teach or suggest the use of carbohydrate-free transferrin (CFT). As is disclosed by Applicant on page 7, lines 14-22, CFT is "carbohydrate-free", that is substantially free of carbohydrate-containing transferrin molecules, i.e. having no carbohydrate side chains and is substantially free of any residual N-linked oligosaccharide moieties, whereas CDT is a carbohydrate deficient transferrin which has varying levels of carbohydrate-containing transferrin molecules. Furthermore, differentiating CFT from other transferrin isoforms according to the present invention is readily accomplished by contacting a body fluid sample with a carbohydrate-binding ligand, specified in step a), to enable separation, as specified in step b), of claim 1). It is not necessary to use a fractionation step as disclosed by Sundrehagen to obtain transferrin isoform separation in the present invention. Instead it can be accomplished by, for example, precipitation.

The Examiner alleged that Applicants have not shown any structural difference between transferrin isoforms nor a substantial absence of carbohydrate by lack of any detectable binding lectins or other carbohydrate binding proteins to CDT. The Examiner's attention is respectfully directed to the specification at page 23, line 35, bridging to page 24, line 5, and the corresponding working Examples 7 and 8 which provide results which were used to generate Figures 1 and 2. Specifically, the results demonstrate using, for example a mixture of immobilized lectins as in Example 7, that the present method can indeed provide CFT material which is free of other carbohydrate containing transferrins.

The Examiner indicated that the present invention with an unrestricted recitation of "separation" reads on Sundrehagen's teaching of fractionation for separation of CDT's which conclusion Applicant disagrees. Applicant's claim 1, step (b), recites:

> (b) separating a carbohydrate-free transferrin containing fraction not binding to said ligand and contacting the separated fraction with an anti-transferrin antibody or an anti-transferrin antibody fragment;

which is believed to distinguish over Sundrehagen since the present process provides separation of unbound CFT from ligand-bound material which is a feature and result not disclosed by Sundrehagen.

With respect to the kit claims 10-12, the above remarks also apply in view of their incorporation of the method of claim 1. The kit claims are further distinguished over Sundrehagen in that, for example, the present invention employs one or more carbohydrate-binding ligands to afford CFT and bound products which can be readily separated according to known techniques such as those mentioned in the specification and in view of user preferences. In contrast, Sundrehagen employs labeled proteinaceous binding partners to form complexes with a proteinaceous analyte variants (CDT's) to produce a population range or distribution of complexed analyte-partners.

Thus, there are differences between the recited method steps and the overall results provided by the present invention compared to Sundrehagen, thus the claims 1-3, 6-8 and 10-12 are not anticipated by Sundrehagen. Accordingly, withdrawal of the rejection is respectfully requested.

Claim Rejections - 35 U.S.C. §103(a)

7. The Examiner rejected claims 4-5 under 35 U.S.C. § 103(a) as being unpatentable over Sundrehagen in view of Pekelharing et al., Analytical Biochemistry, 165, 320 (1987) ("Pekelharing"). The rejection is respectfully traversed.

Applicant's method claim 4, which incorporates and depends from claim 1, is directed toward using "more than one" lectin type (i.e. two or more) as a carbohydrate-binding ligand in the contacting step a), that is, "wherein in step (a) a panel of more than one type of lectin is used as a carbohydrate-binding ligand."

Applicant's method claim 5, which incorporates and depends from claim 1, provides a selection of various useful carbohydrate-binding ligands and mixtures thereof for accomplishing the carbohydrate-binding contacting step (a).

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the cited references themselves or in the knowledge generally available to an art worker, to modify the reference or to combine reference teachings so as to arrive at the claimed invention. Second, the art must provide a reasonable expectation of success. Finally, the prior art reference(s) must teach or suggest all of the claim limitations. The teachings or suggestions, as well as the expectation of success, must come from the prior art, not applicant's disclosure. M.P.E.P. § 2142.

A fair reading of the Examiner's obviousness argument is that one skilled in the art would first, modify an ELISA by replacing the immobilized antibody or enzyme linked antibody with a lectin or other carbohydrate binding protein as taught by Pekelharing; and second, modify the method of Sundrehagen by using (substituting) the modified ELISA to assess alcohol consumption. The Examiner implies that there is motivation to modify and substitute as above to achieve the present invention by asserting 'because Pekelharing teaches the use of lectins . . . ... [improves] an immunoassay'.

The primary reference (Sundrehagen), as discussed above and incorporated herein in its entirety, does not teach or suggest a method for the determination of CFT for use in the assessment of elevated alcohol consumption as in the present invention.

The secondary reference (Pekelharing) does not cure the deficiencies of Sundrehagen. Pekelharing describes a lectin-enzyme immunoassay for determining protein glycosylation, for example, in a mixture of transferrin

sialovariants, such as 4-sialo-transferrin, which upon treatment with neuraminidase produces isoforms having 3, 2, 1 and 0 sialic acid groups (see left-column, page 323 of Pekelharing). The present invention can be accomplished without neuraminidase since CFT present in an alcohol abuser contains no N-glycan chains, see for example working Example 8. Thus, Pekelharing does not teach or suggest a method for determining CFT in alcohol consumption. Additionally, even if one were to combine the references as suggested by the Examiner there would still be no instant method for the determination of carbohydrate-free transferrin (CFT) in a body fluid for use in the assessment of elevated alcohol consumption, nor is there provided a kit which solves the problem addressed in present invention of assessing elevated alcohol consumption by determining CFT.

The Examiner's basis for the obviousness rejection of claims 4 and 5 is therefore believed to be conclusory since neither Sundrehagen alone or in combination with Pekelharing teach or suggest a method for the determination of CFT in a body fluid for use in the assessment of elevated alcohol consumption. Efficiency of the method of Pekelharing combined the method of Sundrehagen does not provide the claimed invention. The Examiner's basis of "expected results" for the required "reasonable expectation of success" fails because it is circular, or alternatively, because the Examiner's "expected results" teach away from the present invention since the method of the primary reference (Sundrehagen) determines variants of CDT and not CFT. The references do not teach or suggest all of the claim limitations such as determining the CFT content. Finally, it appears that the teachings or suggestions to modify or combine, and the expectation of success do not come from the cited documents. Hence, claims 4-5 of the present invention are not obvious over Sundrehagen in view of Pekelharing. Accordingly, withdrawal of the rejection under 35 U.S.C. § 103(a) is respectfully requested.

8. The Examiner also rejected claim 9 under 35 U.S.C. § 103(a) as being unpatentable over Sundrehagen in view of Dreher et al. ("Dreher" Canadian Patent No. 2,074,345). The rejection is respectfully traversed.

Applicant's method claim 9, which incorporates and depends from claim 1, is directed toward determining transferrin content in claim 1 step (c) by turbidometric or nephelometric means.

The primary reference (Sundrehagen), as discussed above and incorporated herein, does not teach or suggest a method for determining CFT for use in the assessment of elevated alcohol consumption.

The secondary reference (Dreher) discloses methods and agents for turbidimetric and nephlometric determination of analytes in liquids with the aid of an antibody-binding reaction, such as binding a polypeptide with an antibody or antibody fragments (thereof), incubating the resulting coupling product with an analyte, and then measuring the resulting turbidity.

The Examiner's obviousness argument appears to be that: 'a routineer would know to use, i.e. substitute, immunotubidimetry and immunonephelometry taught by Dreher in the transferrin assessment method of Sundrehagen because these techniques can be automated.' The Examiner's obviousness basis rejection of claim 9 is believed to be conclusory since neither Sundrehagen alone or in combination with Dreher teach or suggest a method for the determination of CFT in a body fluid for use in the assessment of elevated alcohol consumption and which determination is achieved by turbidimetric and nephlometric means. That the method of Dreher can be simply and easily automated does not provide a basis combining the references. Even if the references were combined as suggested by the Examiner the combination would not provide the present invention since there is no direction or instruction with particularity for the determination of CFT as in the present invention. The Examiner has not provided a reasonable basis for the 'reasonable expectation of success' obviousness prong. The references do not teach or suggest all of the claim limitations such as detecting CFT or determining the content of CFT. Finally, it appears that the teachings or suggestions to modify

or combine, and the expectation of success do not come from the cited references themselves.

For at least the above reasons claim 9 is not obvious over Sundrehagen in view of Dreher et al. Accordingly, withdrawal of this rejection under 35 U.S.C. § 103(a) is respectfully requested.

Conclusion

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney (612-357-3270) to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

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CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Commissioner of Patents, Washington, D.C. 20231, on this 2nd day of December, 2002.

PATRICIA A. HULTMAN
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Signature

CLEAN VERSION OF PENDING CLAIMS

1. (Amended) A method for the determination of carbohydrate-free transferrin in a body fluid for use in the assessment of elevated alcohol consumption, said method comprising

(a) contacting a sample of said body fluid with a carbohydrate-binding ligand, to bind any carbohydrate or carbohydrate-containing moieties in said sample to said ligand;

(b) separating a carbohydrate-free transferrin containing fraction not binding to said ligand and contacting the separated fraction with an anti-transferrin antibody or an anti-transferrin antibody fragment; and

(c) determining the content of carbohydrate-free transferrin in said fraction and thereby determining the content of carbohydrate-free transferrin in said sample.

2. A method as claimed in claim 1, wherein the sample is blood or obtained from blood.

3. (Amended) The method as claimed in claim 1, wherein the carbohydrate-binding ligand is selected from the group consisting of antibodies, antibody fragments , lectins, mammalian carbohydrate-binding proteins, microbial carbohydrate-binding proteins, and mixtures thereof.

4. (Amended) The method as claimed in claim 1, wherein in step (a) a panel of more than one type of lectin is used as a carbohydrate binding ligand.

5. (Amended) The method as claimed in claim 1, wherein the carbohydrate-binding ligand is selected from the group consisting of *Sambucus nigra* lectin, *Sambucus sielbodiana* lectin, wheatgerm agglutinin, *Maackia amurensis* lectin, *E.*

coli K99 lectin, *Helicobacter pylori* lectin, *Ricinus communis* lectin, *Crotalaria junctae* lectin, anti-sialic acid antibodies, and mixtures thereof.

6. (Amended) The method as claimed in claim 1, wherein the separation step (b) is by precipitation, centrifugation, filtration or chromatographic methods.

7. (Amended) The method as claimed in claim 1, wherein the carbohydrate-binding ligand is immobilized.

8. (Amended) The method as claimed in claim 1, wherein an ion exchange step to remove or deplete carbohydrate-carrying transferrins in the sample is performed prior to step (a).

9. (Amended) The method as claimed in claim 1, wherein determining the transferrin content in step (c) is achieved by turbidometric or nephelometric means.

10. (Amended) A kit for use in a method as defined in claim 1, said kit comprising:

one or more carbohydrate-binding ligands;

means for separating unbound carbohydrate-free transferrin from ligand-bound carbohydrate-containing transferrin; and

means for determining the carbohydrate-free transferrin content in the separated portion which determines the content of carbohydrate-free transferrin in the sample.

11. (Amended) The kit as claimed in claim 10, wherein said means for determining the carbohydrate-free transferrin content comprises an anti-transferrin antibody or an anti-transferrin antibody fragment; and optionally an opacification enhancer.

12. (Amended) The kit as claimed in claim 10, further comprising a carbohydrate-free transferrin solution of known concentration or a set of such solutions having a range of carbohydrate-free transferrin concentrations.

13. A method for the detecting carbohydrate-free transferrin in a body fluid for use as an indicator of alcohol abuse, said method comprising

(a) contacting a sample of said body fluid with an immobilized carbohydrate-binding ligand to bind any carbohydrate-containing moieties in the sample to the immobilized ligand;

(b) separating any unbound carbohydrate-free transferrin from any bound carbohydrate-containing moieties;

(c) contacting any separated carbohydrate-free transferrin with an anti-transferrin antibody or an anti-transferrin antibody fragment to form a conjugate; and

(d) detecting the presence of any carbohydrate-free transferrin anti-transferrin antibody conjugate by tubidometry or nephalometry.

14. The method of claim 13, wherein the presence of any carbohydrate-free transferrin is indicative of alcohol abuse.

15. The method of claim 13, wherein the method is free from the influence of amino acid sequence polymorphism in the polypeptide backbone of an abuser's transferrin.

16. The method of claim 13, wherein the method is independent of the abuser's race.

17. A kit for use in a method of claim 13, the kit comprising:

one or more carbohydrate-binding ligands;

means for separating unbound carbohydrate-free transferrin from bound carbohydrate-containing transferrin; and

means for detecting any carbohydrate-free transferrin.